TITLE: Transmission of *Enterocytozoon bieneusi* between a child and guinea pigs.

RUNNING TITLE: Zoonotic transmission of microsporidia

AUTHORS

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ABSTRACT

An unusual Enterocytozoon bieneusi genotype was found in seven guinea pigs and a two-year-old child in the same household. The genetic uniqueness of the parasite and its wide occurrence in other guinea pigs and absence in other children in the community suggest the occurrence of zoonotic transmission of the infection in the study child.
Microsporidia are intracellular organisms that infect humans and animals, and are now considered to be fungi (15). Of the 14 microsporidian species pathogenic to humans, *Enterocytozoon bieneusi* is the most common (4). Initially described in 1985 as a significant pathogen in AIDS patients (5), *E. bieneusi* also affects other immunocompromised populations (10), as well as travelers, children, and the elderly throughout the world (1, 14, 21, 25). While immunocompetent persons often have mild or self-limiting disease, AIDS patients experience chronic diarrhea with accompanying weight loss and increased mortality (23). Antiretroviral treatment is highly effective in reducing the incidence of microsporidiosis in AIDS patients (16). However, few in the developing world have access to this treatment (9). As no antiparasitic therapy has been approved for *E. bieneusi* infection, understanding its transmission mechanisms is crucial for preventing infections (7, 8, 17).

The zoonotic potential of microsporidia was first suggested in 1995 when *Encephalitozoon cuniculi* from rabbits or dogs was reported to be potentially infectious to humans (6). *Enterocytozoon bieneusi* has been reported in several species of domestic and wild animals including dogs, cats, pigs, and cattle (4). Recent surveys found that pigs and park pigeons had genotypes of *E. bieneusi* that were potentially infectious to humans (2, 11). Cattle in the US and other countries were primarily infected with host-specific *E. bieneusi*, but also had some genotypes previously found in humans, thus, potentially zoonotic (18, 20). In total, six genotypes of *E. bieneusi* have been independently found in both animals and humans (13, 19) and all are genetically related to a group of genotypes considered to have broad host-specificity (Figure 1).

We report here the finding of a very unusual *E. bieneusi* genotype in a child, which seems to be a unique parasite of guinea pig origin. This finding was from a prospective pediatric cohort
study of enteric parasites conducted in Pampas de San Juan, Peru, from March 2002 to March 2006. This study was approved by the IRBs from the Centers for Disease Control and Prevention, Johns Hopkins University, and Universidad Peruana Cayetano Heredia. As part of the study procedures, we collected weekly stool specimens from 388 children for microscopy detection of gastrointestinal parasites, daily data on clinical manifestations, and monthly anthropometric measurements. Microsporidia spores were microscopically detected using the Weber-modified trichrome stain (24). Positive specimens were preserved in 2.5% potassium dichromate for genotyping.

Microsporidiosis in a child and household guinea pigs. In June 2005, we identified by microscopy microsporidia spores in the stool of a 25-month-old male participant. Follow-up stool specimens were collected to confirm infection. The child was microsporidia-positive over a 10-day period, and remained microsporidia-negative throughout the next 32 weeks in the study. Other gastrointestinal parasites were not detected in the two previous months and during the infection episode. Contacts between children and their household animals are very frequent in this community, which prompted us to analyze stool specimens of all animals in the household: eight guinea pigs, five chickens, two dogs, and two cats. Seven of the eight guinea pigs, all asymptomatic, had microsporidia spores in their feces whereas the other animals were negative.

Enterocytozoon bieneusi genotyping. The microsporidia-positive stool specimens were genotyped using sequence analysis of the internal transcribed spacer (ITS) of the rRNA gene of E. bieneusi, the only microsporidia species previously found in this study area (3). Briefly, DNA was extracted, the rRNA gene containing the ITS was amplified by PCR (19), and the three independently amplified products were sequenced in both directions for each positive specimen. Sequences were aligned using ClustalX (http://www.ebi.ac.uk/clustalw/) and analyzed for
phylogenetic relationships using the neighbor-joining method in TreeConW software (http://bioinformatics.psb.ugent.be/software) based on the genetic distances calculated with the Kimura 2-parameter model.

**Shared unique *E. bieneusi* genotype in the child and guinea pigs.** All microscopy-positive specimens from the case-child and guinea pigs in the household were positive for *E. bieneusi* by nested PCR. The sequence analysis revealed that the case-child and all guinea pigs had the same genotype (GenBank Accession number EF014427) that was named Peru 16. This genotype was genetically very different from other known *E. bieneusi* genotypes in humans, and was placed outside the cluster of genotypes considered to have broad host-specificity in phylogenetic analysis of the ITS sequences. *Enterocytozoon bieneusi* infection was identified in over 30 children in other households during the study period. They had genotypes Peru 1-15 and Peru 17, which belong within the cluster containing all known human-pathogenic *E. bieneusi* genotypes (Figure 1).

**Occurrence of the unique *E. bieneusi* genotype in guinea pigs in other households.** To assess whether guinea pigs were the natural hosts of Peru 16, we examined stools from additional 59 guinea pigs in 20 households whose participants were microsporidia negative. Three guinea pigs from two unrelated households in the study had this unusual genotype, suggesting that guinea pigs were the natural hosts of Peru 16.

**Clinical manifestations of the *E. bieneusi* infection.** The case-child participated 76-weeks in the study. During that period, the child had a total of three episodes of diarrhea (≥3 liquid or semi-liquid stools per day) at ages 17-months (3 days), 23-months (1 day), and on the first day of this infection-episode. He had an average weight-for-height Z-scores of -0.29 at the onset of symptoms, which was comparable to the nutritional status of other 24-month-old
children in the study (n=260, mean Z-score of -0.13). However, this child suffered weight loss around the symptomatic infection episode, followed by a decline in Z-scores in the months following infection (Z= -1.04, -1.33, and -1.11 for May, June, and July of 2005, respectively). This drop started almost concurrently with the onset of symptoms and the episode of diarrhea at the beginning of this infection-episode. Follow-up measurements indicated no further declines in Z-scores at six, seven and eight months post infection (Z=-0.83, -1.28 and -1.36, respectively), time at which the child withdrew from the study. No catch-up growth was observed. This finding is in contrast to previous reports where no associations were found between being underweight and *E. bieneusi* infections (12, 22). Alternatively, this infection with Peru 16 and weight loss could have been indicators of other underlying conditions.

**Zoonotic potential of *E. bieneusi***. The uniqueness of Peru 16 (its location in a clade independent of other *E. bieneusi* genotypes previously reported in humans), the finding of this genotype in guinea pigs from unrelated households, and the close contacts between the case-child and infected guinea pigs all strongly suggest that child probably acquired the infection from guinea pigs in the household. This finding indicates that even some *E. bieneusi* genotypes seemingly unique in some animals have zoonotic potential. Because the child showed no evidence of immunosuppression during the longitudinal fellow-up and the prevalence of HIV is low in the community (26), the finding of diarrhea in the child at the time of symptomatic infection suggests that *E. bieneusi* may cause short-lived gastrointestinal manifestations in immunocompetent persons. Because this infection episode was short and self-limiting, it is likely that *E. bieneusi* infections are underreported in the general population.

While this study shows that the guinea pig is likely the natural host for *E. bieneusi* genotype Peru 16, more studies are needed to determine whether the previously reported human
infections with genotypes C and Q, which are also genetically unique, were due to zoonotic
transmission, whether other animal species are capable of transmission to humans, and what
specific risk factors favor zoonotic transmission.

This work was supported in part by grants from NIH-NIAID Peru-TMRC 5P01AI051976, R21
AI 059661, and USDA-CSREES number: 2001-51110-11340. The findings and conclusions in
this manuscript are those of the authors and do not necessarily represent the views of the Centers
for Disease Control and Prevention.
REFERENCES


FIGURE LEGENDS

Figure 1: Phylogenetic relationships between *E. bieneusi* genotype Peru 16 and other genotypes previously described in humans and animals. Percentage bootstrap values (>50%) from 1,000 replicates are presented to the left of the nodes.
Genotypes previously reported in humans or animals